COMPREHENSIVE FINAL REPORT TO THE OFFICE OF NAVAL RESEARCH

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rincipal Investigator: Felix Haurowitz, M.D., D.Sc., Distinguished Service Professor, Department of Chemistry, Indiana University, Bloomington, Indiana.
oworkers: Drs. G. Vidaver, E. F. Gold M. A. Lopez, ". Ozkan; and the following graduate students: Magda Groh, Kay Knight, Grant Gansinger, J. L. Groff, H. Stine and L. F. Romain.
itle of Project: Mechanism of Antigen-Antibody Reactions and Similar Nonspecific Reactions between Proteins and Other Macromolecules.
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## RESULTS

ersistence of Antigen. In the early work from our laboratory the problem of the ersistence of the antigen in the antibody forming organs was investigated because : was not clear at that time whether the antigen is necessary only for the itiation of antibody formation or whether it is also necessary for continued tibody formation. Relying on the stability of the iodine label in circulating dinated protein antigens, several authors claimed that the iodine label is also able in the tissues. Using doubly-labelled proteins containing $^{131}I$ and either S- or $^{14}C$-haptens, we found that $^{35}S$-azophenylsulfonate and $^{14}C$-azobenzoate ptens of the doubly labelled proteins persists much longer than the $^{131}I$-label. idently, the doubly labelled proteins are deiodinated in the tissues although they main iodinated as long as they circulate in the blood plasma. Consequently, radiotive iodone cannot be used as an indicator of the persistence of the carrier protein tigens. Experiments with $^{35}S$- and $^{14}C$-azoproteins indicate an initial rapid dease, followed by a much slower decrease in the concentration of the isotopes in leen and liver. In this second phase of antigen loss the biological halflife of e used haptens in the spleen is approximately 1-2 months. At this rate significant ounts of antigen would be present in the tissues even after 1-2 years. This would plain the continued production of antibodies over periods of years.

amnestic (Secondary) Response. The typical "anamnestic reaction" produced by rejection of an antigen is frequently attributed to a "memory" of the cell for the rst injection. Our results described in the preceding section suggested that the amnestic reaction is caused by immediate combination of reinjected antigen with ntinuously formed antibody. To test this hypothesis we investigated the serum of bbits sensitized by a single injection of bovine serum albumin (BSA) or ovalbumin A) for the presence of antibody. Using the passive hemagglutination technique ich is at least 1000 times more sensitive than the precipitin test, we found ined continuous formation of antibody over periods of at least one year, even after single injection of the antigen. Reinjection, in contrast to the first injection, ads to immediate combination of the reinjected antigen with the homologous antiy in the circulation and with the cells which form this antibody and secrete it to the blood plasma. The antigen-antibody complexes formed in this almost mediate reaction stimulate antibody formation much more than the soluble antigen lecules used in the primary sensitization. According to our results, the "memory lls" of other authors are merely cells which still continue to form small amounts antibody and which, therefore, bind strongly the reinjected antigen.



ibody Structure . Porter (1959) and Edelman and his co-workers (1961) have shown t the typical 7S antibody of rabbits and man consists of 2 light (L) chains and heavy (H) chains. The work of the two laboratories raised the hope that more

information on antibody structure could be gained by the comparative analysis of antibodies of different serological specificity. We decided to compare the hapten-specific antibody directed against the p-azophenylarsonate anion with an analogous antibody against the p-azophenyl-N-trimethylammonium cation. The two haptens were coupled to BSA. Antibodies were isolated from the immune sera by means of columns of aminobenzylcellulose coupled with the same haptens. The antibodies, designated as anti-As and anti-$R_4N$, were separated from each other and also from anti-BSA formed in the same animals. We obtained the same two antibodies also from rabbits injected with doubly substituted As-$R_4N$-BSA. In using this doubly labelled antigen we avoided complications arising from differences in the genetic type of the injected animals; moreover, we could be sure that both haptens were initially carried to the same cells. We compared the peptide maps of the isolated antibodies, the column chromatograms of their trypsin digests, and their electrophoretic behavior. We found, however, no consistent differences between anti-As and anti-$R_4N$. Since the two antibodies are certainly different, and since they refold after unfolding spontaneously yielding active antibody, as shown by Tanford and his co-workers and by Haber, we must conclude that the peptide spots and peptide peaks produced by the digests of anti-As and anti-$R_4N$ are those of peptides which occur in both antibodies; we further conclude that those amino acid sequences which form the specific combining sites are heterogeneous, each variant occurring in too small concentrations to give a peptide spot on the paper or a peak on column chromatography. Heterogeneity of the L chains is clearly demonstrated by our starch gel electrophoreses in which we obtain 6-8 bands for the L chain of serologically pure anti-As or anti-$R_4N$ antibodies. The observed heterogeneity of serologically pure antibodies forces us to abandon the view that a well-defined antigenic hapten induces the formation of only one type of well-defined combining site in the homologous antibody. Evidently, different amino acid sequences can yield combining sites of very similar conformation, each of these conformations closely fitting the surface of the homologous haptenic determinant group. None of the theories on antibody formation has taken this variability of antibody molecules into consideration. Therefore, a new approach is necessary. On invitation of numerous scientific organizations, the principal investigator has written several review articles on this fundamental problem of immunology.

It may, finally be mentioned, that the principal investigator received a series of new invitations to present lectures or to chair meetings on problems of immunochemistry. During the year 1965 and the first 6 months of 1966 lectures were presented at the University of California Medical School in San Francisco, the Karolinska Institute in Stockholm, The Institute f. Physical Chemistry at Uppsala (Sweden), the Immunological Society in Amsterdam (Holland), the Weizmann Institute in Rehovoth, the Universities of Jerusalem, Tel-Aviv, Istanbul and Smyrna (Izmir), and Duke University (2 lectures). I also acted as session chairman in a Conference on Hemoglobin in Cambridge (England), at a meeting on Antibody Response Regulation at Toronto (Canada), at a Conference on Immunoglobulins in Gatlinburg (ORNL) and at a Gordon Research Conference on Antigens (Crystal Mountain, Washington). The travel expenses for all these trips were carried by the inviting organizations. None of these trips was paid from the ONR contract. The numerous invitations seem to indicate a great interest in the U. S. and in overseas in our work which has been supported so generously by the Office of Naval Research.

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